Repair of DNA Damage Induced in Systemic Lupus Erythematosus Skin Fibroblasts by Simulated Sunlight

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Skin fibroblasts derived from three normal individuals and three patients exhibiting the disease systemic lupus erythematosus (SLE) were exposed to the simulated sunlight produced by a solar simulator. The induction and repair of DNA damage induced by this treatment were examined. The total number of lesions repaired by excision, as well as the removal of pyrimidine dimers and *E. coli* endonuclease III – sensitive sites did not differ significantly in the three SLE cell strains compared with normal cells. However, abnormalities in the formation and maintenance of DNA-protein crosslinks (DPC) and DNA single-strand breaks (SSB) were found in SLE-4 and SLE-5 following simulated sunlight exposure. In contrast, SLE-3 cells exhibited responses similar to normal cells in reference to SSB and DPC formation. These findings correlate well with the previously determined UV sensitivity of these SLE cell strains. *J Invest Dermatol 98:469–474, 1992*

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the involvement of multiple organs and the production of a variety of antibodies reactive with many cellular components. Hyperactive B lymphocytes and functional abnormalities of T lymphocytes appear to be responsible for the abundance of autoantibodies found in the sera of SLE patients [1]. The synthesis of antibodies against native DNA has been considered relatively specific for SLE, and immune complexes containing DNA appear to play an important role in the pathogenicity of SLE [2,3].

In many SLE patients, the development of cutaneous lesions and systemic symptoms are provoked by sunlight. The incidence of photosensitivity has generally been reported to be between 40 and 60%, though this figure varies depending upon the population of SLE patients examined [4,5]. Cutaneous lesions have also been elicited by exposure to UV radiation under controlled laboratory conditions [6,7]. Recent investigations indicate that the UV-induced transposition of the small ribonucleoprotein SSA/Ro to the plasma membrane of epidermal keratinocytes may be an important component of the cutaneous response to solar ultraviolet radiation in SLE patients who express anti-SSA/Ro autoantibodies [8,9]. A role for immune complexes containing DNA and anti-DNA antibodies has also been suggested [10].

Despite the frequency of photosensitivity in SLE, the mechanism by which the UV component of sunlight alters the pathogenic course of the disease is poorly understood. We have proposed that the persistence of UV-induced DNA damage caused by repair deficiencies, in coexistence with the immunoregulatory dysfunctions found in SLE, may result in enhanced levels of pathogenic immune complexes containing DNA [11]. Very little is known about the DNA repair capacity of cells from patients with SLE. Beighlie and Teplitz found that a lower percentage of UVC-irradiated SLE lymphocytes incorporated ^{3}H-thymidine than normal lymphocytes [12]. Although it was suggested that this difference was caused by a repair deficiency, such an interpretation of the data has been challenged [13]. Harris et al have suggested that the increased sensitivity of SLE lymphocytes to N-methyl-N-nitrosourea is due to an inability to repair 0^6-methylguanine sites [14]. Increased levels of spontaneous chromosome aberrations and sister chromatid exchanges, as well as increased UV toxicity, in SLE lymphocytes [15–17] are also suggestive of DNA-repair deficiencies. Zamansky et al have investigated the in vitro photosensitivity of SLE skin fibroblasts and found that four of five cell strains were hypersensitive to UV radiation as measured by cellular lethality [11]. UV-sensitive SLE cells had a normal capacity to repair potentially lethal damage [18] and to perform unscheduled DNA synthesis [11] following UV irradiation. This is an agreement with D'Ambrosio et al (1983) who found that pyrimidine dimers were removed normally in cells from SLE patients UV irradiated in vivo [19].

In the current study we have examined the ability of three SLE cell strains to repair DNA damage induced by simulated sunlight. We have found that SLE cells are able to repair normally several types of DNA lesions. However, UV-sensitive SLE cell strains are unable to maintain a normal level of DNA-protein crosslinks (DPC) or DNA single-strand breaks (SSB) following exposure to simulated sunlight. These results represent an important demonstration of a specific abnormality in the ability of SLE cells to process sunlight-damaged DNA.

MATERIALS AND METHODS

Cell Strains Normal human skin fibroblast cell strains were obtained from the Human Genetic Repository (Institute for Medical Research, Camden, NJ). Cell strain GM 4390 was derived from the forearm of a 23-year-old female, GM 3468 from the foreskin of a 5-day-old male, and GM 5386 from fetal skin tissue of a 20-week post-gestation male fetus. As described previously [11], the SLE cell strains were derived from the medial forearm of SLE patients who

Manuscript received February 8, 1991; accepted for publication August 12, 1991.

This research was supported by NIEHS grant ES04355 and NCI grant CA45078, DHHS.

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Abbreviations:
BrdUrd: bromodeoxyuridine
DPC: DNA-protein crosslinks
PBS: phosphate-buffered saline
SDS: sodium dodecyl sulfate
SLE: systemic lupus erythematosus
SSB: DNA single-strand breaks

0022-202X/92/$05.00

fulfilled four or more of the American Rheumatism Association criteria for the classification of SLE. SLE-3 was obtained from a 45-year-old man, SLE-4 from a 23-year-old man, and SLE-5 from a 40-year-old woman. All cell strains were used before reaching cell passage 15.

The UV sensitivities of the SLE cell strains utilized in the current study have previously been reported [11]. Briefly, SLE-3 displays normal survival parameters following exposure to UV, whereas SLE-4 and SLE-5 are hypersensitive to UV radiation. For example, following sunlamp UV irradiation, the D_0 values (dose required to reduce survival to 37% in the exponential portion of a survival curve) for three normal cell strains were 310.4 ± 16.9, 341.9 ± 19.8, and 347.6 ± 23.8 J/m^2. The D_0 values for SLE-3, SLE-4, and SLE-5 were 311.0 ± 7.7, 256.9 ± 3.3, and 208.6 ± 5.7 J/m^2, respectively. These values may also be compared to the D_0 of a xeroderma pigmentosum (complementation group C) cell strain, which had a D_0 of 141.5 ± 3.9 J/m^2.

Culture Conditions Cells were grown in Eagle's minimum essential medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (Hyclone, Logan, UT), 100 units/ml penicillin and 100 μg/ml streptomycin, 2 mM L-glutamine, 2× amino acids solution, 2× non-essential amino acids solution, and 2× vitamin solution (GIBCO). Cultures were incubated at 37°C in a 5% CO_2 humidified atmosphere. Under these conditions all of the cell strains utilized had a doubling time of 1–2 d.

Irradiation Conditions Prior to each irradiation cells were washed three times with phosphate-buffered saline (PBS [20]), covered with 5 ml of PBS, and exposed to the simulated sunlight produced by an Oriel 1000-watt Solar Simulator at a fluence rate of 1.7 kJ/m^2/second while being held at approximately 4°C on ice. The fluence rate was measured using a IL1700 Research Radiometer (International Light, Newburyport, MA). The output of the lamp was passed through a 1.5 Air Mass filter in order to simulate the sunlight spectrum at the earth's surface [21].

Measurement of DNA-Protein Crosslinks (DPC) and DNA Single-Strand Breaks (SSB) Cells were plated in either 60-mm dishes or 75-cm^2 flasks (Corning Glass Works, Corning, NY) at a density of 10^4 cells/cm^2 and either methyl-[^{3}H]-thymidine (20 Ci/mmol, New England Nuclear, Boston, MA) or 2-[^{14}C]-thymidine (59 mCi/mmol) was added to a final concentration of 0.1 μCi/ml or 0.02 μCi/ml, respectively. Following a 72-h incubation, the ^{14}C-labeled cells were exposed to the simulated sunlight and incubated 0–24 h. The cells were then washed twice with PBS and gently scraped off the flasks into ice-cold PBS containing 0.2 mg/ml Na_2EDTA. Aliquots containing 5×10^5 ^{14}C-labeled cells exposed to simulated sunlight were mixed with 5×10^5 ^{3}H-labeled cells and irradiated with 10 Gy of x-rays (250 kV Phillips Model RT 250 at a dose rate of approximately 1.1 Gy/min) while held on ice for measurement of DPC [22]. In addition, for each treatment, 5×10^5 ^{14}C-labeled cells not exposed to x-ray were mixed with 5×10^5 ^{3}H-labeled cells that had been irradiated with 3 Gy of x-rays in order to determine the level of SSB. Each cell mixture was then loaded on a 25-mm, 2-μm pore size polyvinyl chloride filter (Millipore Corp., Bedford, MA) for determination of DPC and on a 25-mm, 2-μm pore size polycarbonate filter (Nucleopore Corp., Pleasanton, CA) for measurement of SSB. The cells were then washed twice with cold PBS and lysed with a solution containing 2% sodium dodecyl sulfate (SDS, Gallard-Schlesinger, Carle Place, NY), 0.1 M glycine, and 0.02 M EDTA, pH 10. The lysis solution was allowed to flow through the filter by gravity. For elutions to measure DPC, the cell lysates were washed with 5 ml of 0.02 M Na_2EDTA, pH 10, whereas for measurement of SSB, 2 ml of lysis solution containing 0.2 mg/ml proteinase K (Scientific Products) was added. In both cases, this was followed by the addition of an elution solution consisting of 0.1 M tetrapropyl-ammonium hydroxide and 0.02 M EDTA (acid form), pH 12.1, and 0.1% SDS. The elution solution was pumped through the filters at approximately 0.03 ml/min and 6 ml fractions were collected at 3-h intervals. Upon completion of the elutions, the fractions were made isovolumetric with water and mixed with 10 ml of Budget-Solve (Research Products International Corp., Mount Prospect, IL) containing 0.3% acetic acid. Filters were processed as previously described [22]. All fractions were counted in a Packard 2000 scintillation counter.

The number of DPC per 10^{10} Da was calculated from the following equation [23]:

$$DPC_{SS} = [(1 - r)^{-1/2} (P_{bR} + P_{SS})] - [(1 - r_0)^{-1/2} (P_bR) + P_{SS}],$$

where DPC_{SS} is the frequency of simulated sunlight-induced DPC, P_{bR} is the frequency of x-ray-induced SSB, P_{SS} is the frequency of simulated sunlight-induced SSB, and r and r_0 are the fractions of DNA eluting in the slow component of the elution profiles extrapolated to time zero for simulated sunlight-treated and untreated cells, respectively.

The yield of SSB induced by the simulated sunlight irradiations was calculated from the following equation:

$$SSB/dalton = 8.1 \times 10^{-10} (B_{SS} - B_{unirr})/(B_{3Gy} - B_{unirr}),$$

where B equals the logarithm of the fraction of DNA retained by the filter after 0 h of elution minus the logarithm of the fraction of DNA retained by the filter after 3 h of elution. The x-ray dose used, 3 Gy, produces approximately 8.1 breaks/10^{10} daltons [24].

Measurement of Pyrimidine Dimers and *E. coli* Endonuclease III–Sensitive Sites Cells were plated in either 60-mm dishes or 75-cm^2 flasks at a density of 10^4 cells/cm^2 and either ^{3}H-thymidine or ^{14}C-thymidine were added to final concentrations of 1.0 μCi/ml or 0.2 μCi/ml, respectively. Following a 72-h incubation, the ^{14}C-labeled cells were exposed to simulated sunlight and incubated 0 or 16 h. The cells were then washed twice with PBS and gently scraped off the flasks into ice-cold PBS containing 0.2 mg/ml Na_2EDTA. Aliquots containing 5×10^4 ^{14}C-labeled cells were mixed with 5×10^4 ^{3}H-thymidine cells that were irradiated with 30 Gy of x-rays. The cells were then lysed on a polycarbonate filter and incubated for 2 h in a buffer composed of 10 mM Tris, 10 mM EDTA, and 100 mM KCl containing 500 units of either purified *E. coli* endonuclease III (kindly supplied by Drs. S. Wallace and N. Duker) or T4 pyrimidine dimer DNA glycosylase-AP endonuclease (Applied Genetics, Freeport, NY). The elutions were then performed as described above for measurement of SSB, except the pump speed was increased to 0.6 ml/min and fractions collected every 10 min. The number of endonuclease-sensitive sites is equal to the number of SSB measured in irradiated cells minus the number of SSB in unirradiated cells.

Excision Repair The overall levels of excision repair were measured in the cell strains using the modified bromodeoxyuridine (BrdUrd) photolysis assay [25]. Briefly, for this assay 7.5×10^5 cells were plated in 100-mm culture dishes and grown in medium containing either 0.05 μCi/ml ^{3}H-thymidine or 0.01 μCi/ml ^{14}C-thymidine for 3 d. The medium was then removed and replaced with medium containing 0.5% fetal calf serum instead of the normally used 10%. These cultures were incubated an additional 3 d and exposed to simulated sunlight. Following irradiation, the ^{3}H-labeled cells were incubated in medium containing thymine and the ^{14}C-labeled cells with BrdUrd for 16 h. The ^{3}H-labeled and ^{14}C-labeled cells for each treatment were then mixed, placed in a 75-cm^2 flask at a concentration of 2×10^5 cells/ml and 2.5-ml samples removed following exposures, while held on ice, to photolytic treatments of $0 - 10 \times 10^{-2}$ breaks/BrdUrd. The photolytic source for these experiments was sunlamp UV that was filtered through the polystyrene culture flask top. These samples were then subjected to alkaline elution under conditions that eliminate any DPC and minimize protein absorption as described for measurement of SSB. The number of SSB induced in the ^{3}H-labeled cells was subtracted from the number of SSB in the ^{14}C-labeled cells, which yields the number of SSB induced through BrdUrd photolysis.

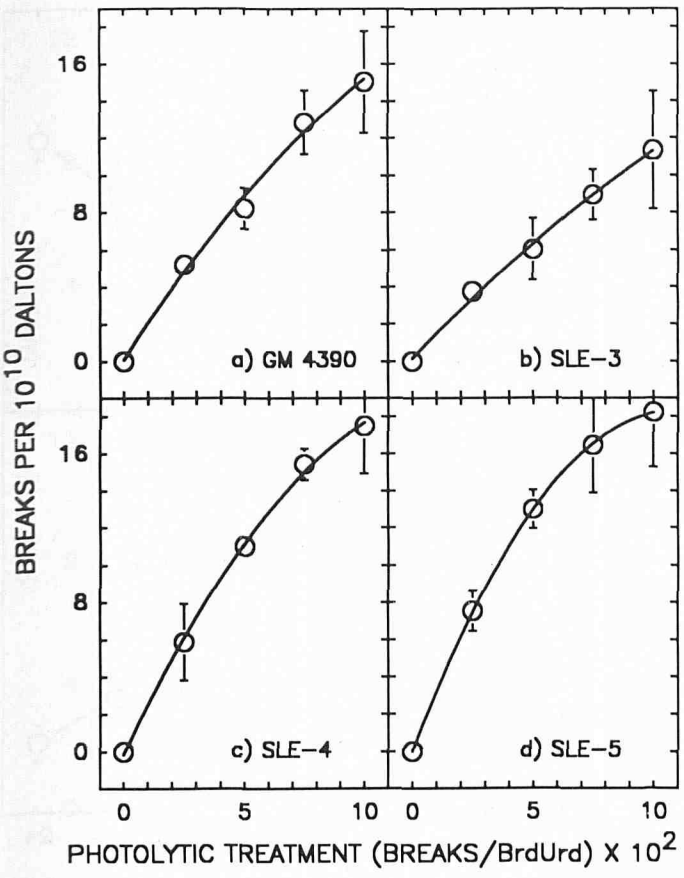

Figure 1. Measurement of excision repair by BrdUrd photolysis. Cells were exposed to 10 kJ/m² simulated sunlight and the number of SSB induced through photolysis of BrdUrd incorporated into parental DNA following a 16-h repair incubation determined. The photolytic treatment represents the fluence of sunlamp UV used to produce photolysis of BrdUrd containing DNA multiplied by the photolytic cross section determined for fully substituted DNA. *Point,* average value for three treatments, *error bars,* standard deviations.

Semiconservative DNA Synthesis Cells were plated at 2.5 × 10^6 per 60-mm dish in medium containing 0.01 μCi/ml of ^{14}C-thymidine and incubated for 3 d. The cultures were then irradiated with either 0 or 100 kJ/m² of simulated sunlight and incubated for either 0, 3, 6, 12, or 24 h. The cells were next incubated 15 min in medium containing 10 μCi/ml of ^{3}H-thymidine. The ratio of ^{3}H to ^{14}C counts in TCA-precipitable DNA was used to determine the kinetics of the inhibition and recovery of DNA synthesis.

RESULTS

Repair Synthesis The overall levels of excision repair were determined using the modified BrdUrd photolysis assay (Fig 1). Each cell strain was exposed to 10 kJ/m² of simulated sunlight and the number of repaired sites as well as the average number of nucleotides inserted per repaired site determined (Table I). The number of damaged sites removed during the 16-h repair period was similar in the SLE and normal cell strains, though SLE-5 exhibited a small increase in the average number of nucleotides inserted into the repaired regions.

Pyrimidine Dimers The normal and SLE cell strains were exposed to 100 kJ/m² of simulated sunlight and the level of pyrimidine dimers measured either immediately after irradiation or following a 16-h incubation. Pyrimidine dimers were detected as sites sensitive to the T4 DNA glycosylase-AP endonuclease, which produces nicks in DNA at sites of these lesions [26,27]. The results of these experiments (Table II) indicate that similar levels of dimers were removed in each cell strain during incubation.

Table I. Overall Levels of Excision Repair

Cell Strain	Repaired Sites per 10^{10} Da[a]	Nucleotides Inserted per Repaired Site[a]
GM 4390	30 ± 14	21 ± 13
SLE-3	24 ± 15	19 ± 18
SLE-4	28 ± 7	30 ± 11
SLE-5	24 ± 4	46 ± 14*

[a] For each treatment, three cultures of cells were exposed to 10 kJ/m² of simulated sunlight and the overall level of excision repair measured using the BrdUrd photolysis assay following a 16-h incubation. The number of repaired sites and average number of nucleotides inserted per site were estimated using the equation $B = N(1-e^{-n\sigma F})$, where B is the number of breaks induced by BrdUrd photolysis, N is the number of repaired sites, n is the average number of nucleotides inserted per repaired site, σ is the photolytic cross section, and F is the sunlamp fluence. The parameters N and n were estimated using PROC NLIN in SAS version 6.03 on a personal computer. The parameter estimates have asymptotic t-distributions and t-statistics were computed to compare the parameter estimates for the SLE cell strains with those of the GM 4390 cells. The values presented represent the averages with standard deviations and differences with $p < 0.05$ are denoted with an *asterisk.*

***E. Coli* Endonuclease III–Sensitive Sites** Cells were irradiated with 500 kJ/m² of simulated sunlight and the level of sites sensitive to the *E. coli* endonuclease III measured using the alkaline elution assay at either 0 or 16 h after irradiation. This enzyme has been shown to be an AP endonuclease with associated N-glycosylase activity [28,29]. It recognizes a variety of thymine ring saturation or fragmentation products [30] and a cytosine photoproduct [31]. The number of damaged sites recognized by this enzyme removed during the 16 h following irradiation did not differ significantly for any of the cell strains (Table II).

Semiconservative DNA Synthesis The kinetics of the depression and recovery in DNA synthesis were investigated in the four cell strains following irradiation with 100 kJ/m² of simulated sunlight. All four cell stains exhibited similar patterns of inhibition of DNA synthesis during the first 6 h post-irradiation and recovery of DNA synthesis to their original levels by 24 h (Fig 2).

DPC and SSB Irradiation of GM 4390 cells with 500 kJ/m² of simulated sunlight resulted in the production of DPC and SSB. The levels of DPC and SSB increased with similar kinetics, reaching maximum levels generally within 9 h, and remained elevated through the 24 h post-irradiation incubation period (Figs 3 and 4). The SLE-3 cells exhibited a similar response to that produced with

Table II. Repair of Pyrimidine Dimers and *E. coli* Endonuclease III–Sensitive Sites

Cell Strain	Percent Remaining Pyrimidine Dimers[a]	Percent Remaining *E. coli* Endonuclease III–Sensitive Sites[a]
GM 4390	21 ± 4	29 ± 18
SLE-3	17 ± 10	23 ± 9
SLE-4	19 ± 14	32 ± 10
SLE-5	10 ± 6	17 ± 10

[a] Entries represent the average percents with standard deviations for either pyrimidine dimers or *E. coli* endonuclease III–sensitive sites remaining following a 16-h incubation after irradiation with either 100 kJ/m² or 500 kJ/m² of simulated sunlight, respectively. Three cultures were irradiated and incubated for each treatment. The level of dimers induced by this fluence in GM 4390, SLE-3, SLE-4, and SLE-5 cells were 10, 12, 10, and 13 per 10^9 Da. The initial levels of *E. coli* endonuclease III–sensitive sites produced in these cell strains were 8, 9, 7, and 8 per 10^9 Da. t statistics were computed to compare each of the three SLE cell strains with GM 4390 for both types of DNA damage. No differences with $p < 0.05$ were detected.

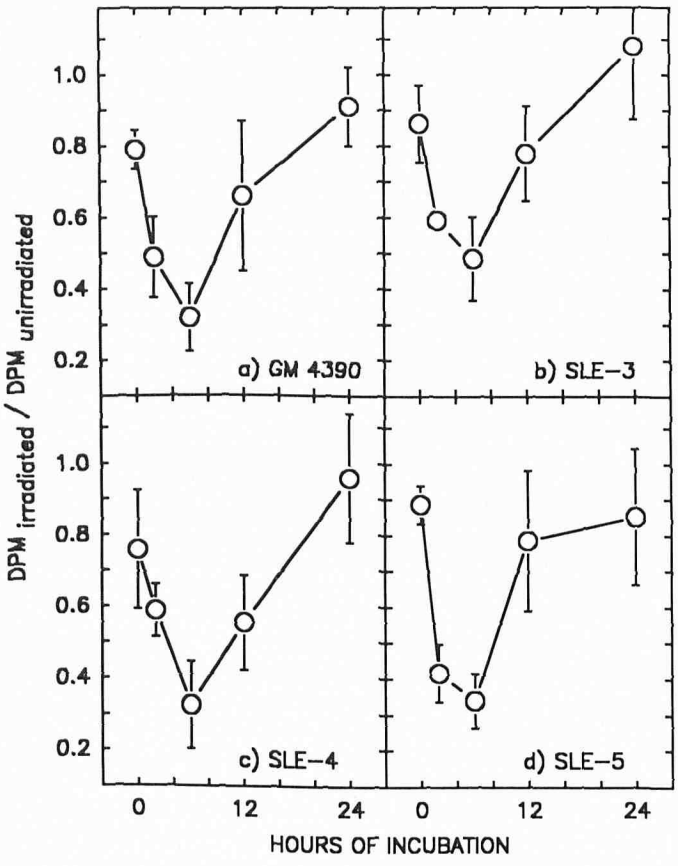

Figure 2. Inhibition and recovery of semi-conservative DNA synthesis. Cultures of each cell strain were exposed to either 0 or 100 kJ/m^2 of simulated sunlight, incubated 0–24 h and pulse labeled with ^{3}H-thymidine. The ratio of DPM incorporated into irradiated cells divided by the DPM incorporated into unirradiated cells is a measure of the inhibition of DNA synthesis. *Point,* average value for three treatments; *error bars,* standard deviations.

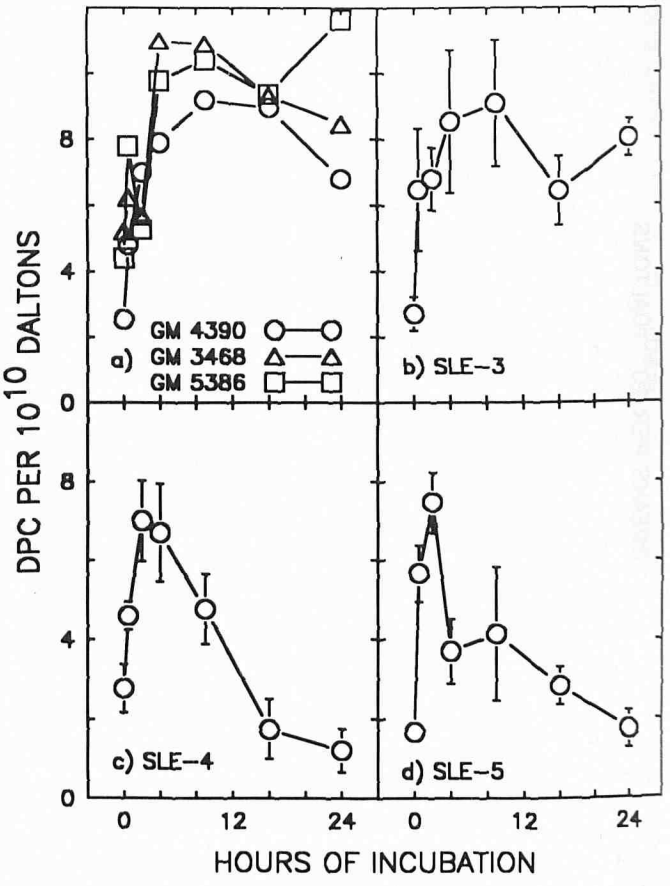

Figure 3. Kinetics of DPC induction. Cells were exposed to 500 kJ/m^2 of simulated sunlight and the levels of DPC determined. *Point,* average value for three treatments; *error bars,* standard deviations. Error bars were not included in *a* as it would not be possible to distinguish between the error bars for the cell strains at each time point.

normal cells. In contrast, the UV-sensitive SLE-4 and SLE-5 strains, although displaying an increase in DPC and SSB during the first 4 h following irradiation, exhibited dramatic decreases in these parameters upon further incubation. By 16 h, both DPC and SSB had declined to levels that were comparable to or lower than those found initially after irradiation. In addition to GM 4390, two more normal cell strains, GM 3468 and GM 5386, were utilized for these experiments in order to confirm the abnormality observed with SLE-4 and SLE-5. The results obtained for these two normal cell strains (Figs 3*a* and 4*a*) were very similar to the responses exhibited by GM 4390.

DISCUSSION

The effects of exposure to simulated sunlight in normal human cells and three cell strains obtained from patients exhibiting the disease SLE were examined. The overall levels of excision repair as well as the removal of pyrimidine dimers and *E. coli* endonuclease III–sensitive sites did not differ significantly in the three SLE cell strains compared with normal cells. In addition, the kinetics of depression and recovery of DNA synthesis were comparable in all four cell strains. The patterns of DPC and SSB formation were also alike in normal and SLE-3 cells, which exhibit similar sensitivities to UV irradiation [11]. However, SLE-4 and SLE-5 cells exhibited abnormalities in the formation and maintenance of DPC and SSB. This inability to properly maintain the level of DPC and SSB following irradiation correlates well with the known UV hypersensitivity of SLE-4 and SLE-5 cells [11].

As has been observed previously in Chinese hamster and human cells exposed to 254 nm UV, an increase in the levels of DPC and SSB were detected following incubation of irradiated cells [32,33]. The protein involved in crosslinking may be part of a DNA-repair process or a cellular response to perturbations in DNA structure. Possible candidates for this protein are type I or type II topoisomerase as these enzymes form covalent linkages to DNA that are associated with either single- or double-strand breaks, respectively [34].

Although SLE-4 and SLE-5 cells initially displayed an increase in the yields of DPC and SSB comparable to levels achieved in normal cells, the numbers of DPC and SSB in these cell strains quickly reached peak values and then decreased dramatically upon further incubation. This is in contrast to DPC and SSB in normal cells in which these parameters remained elevated during the entire course of the experiment. If the rapid decrease of DPC and SSB in the SLE-4 and SLE-5 was indicative of an abnormally rapid repair of DNA lesions induced by irradiation, then the hypersensitivity of these cells to UV radiation would be paradoxical because such DNA repair should enhance cell survival. These results are similar to those that have been obtained with two mutants derived from the ICR 2A cell line, which displayed an abnormality in the formation of DPC and hypersensitivity to solar UV radiation [35]. These combined findings are strongly suggestive that the formation of DPC and SSB are part of a repair process that plays a role in cellular survival.

A critical point of these experiments is that it is important to

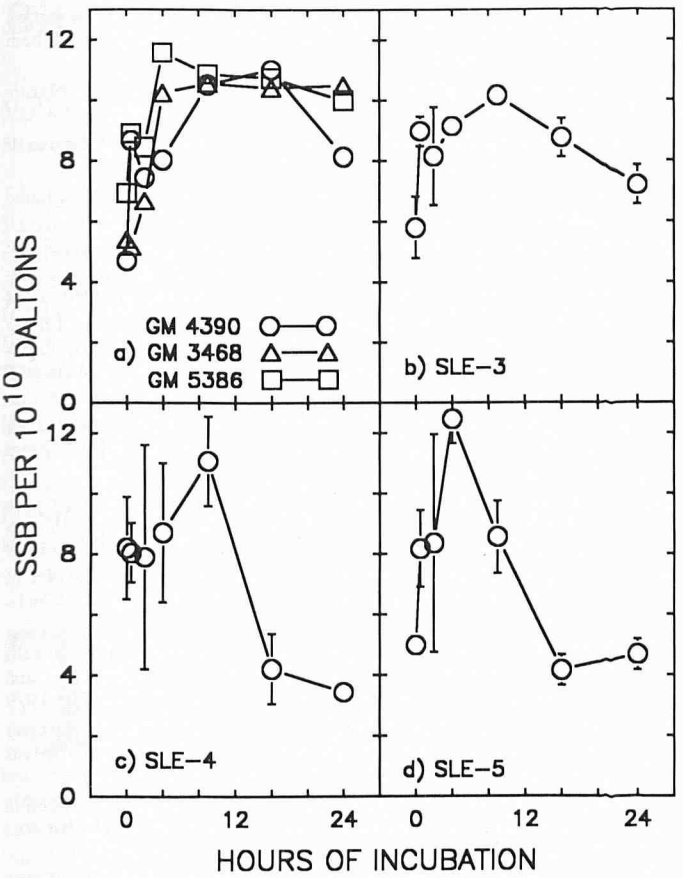

Figure 4. Kinetics of SSB induction. Cells were exposed to 500 kJ/m² of simulated sunlight and the levels of SSB determined. *Point,* average value for three treatments, *error bars,* standard deviations. Error bars were not included in *a* as it would not be possible to distinguish between the error bars for the cell strains at each time point.

distinguish between the DPC and SSB induced directly by the simulated sunlight treatment from the DPC and SSB that are formed by a cellular pathway in response to the irradiation. Immediately after exposure, 2–6 DPC and SSB per 10^{10} Da were generally observed in the normal and SLE cells. However, upon incubation, the number of DPC and SSB continued to increase until a maximum of 8–12 DPC and SSB per 10^{10} Da were detected. Thus, the majority of the total number of DPC and SSB formed were synthesized by the cell following irradiation.

It is interesting to note that for the excision-repair experiments performed using the BrdUrd photolysis assay the average patch sizes determined were 19–46 nucleotides inserted per repaired site. These values are close to those obtained using the CsCl density-shift technique [36] and lower than the 100–200 nucleotides generally reported for patch size when the BrdUrd assay was performed using sucrose gradient sedimentation [37]. Two possible explanations could be offered for this finding. The first is related to an artifact in the sucrose gradient approach that may have led in certain cases to underestimates in the levels of breaks induced by BrdUrd photolysis at high photolytic treatments. This in turn resulted in overestimates in the calculations of patch size [25,38]. Therefore, great care was taken in the performance of the experiments described in this paper to insure accurate estimates for break induction, particularly for high photolytic treatments. A second possible explanation is related to the use of simulated sunlight as the radiation source responsible for the induction of DNA damages. Approximately 94% of the UV radiation produced by the solar simulator used in these experiments falls within the UVA region and the remaining 6% in the UVB range [39]. UVC wavelengths are completely excluded. It has been found in previous work that UVA wavelengths produce damages that are eliminated primarily by a short-patch repair mechanism [25]. In addition, it can be determined from the results presented in this paper that the level of pyrimidine dimers induced by the fluence used for the BrdUrd photolysis experiments was approximately 11/10^{10} Da. However, 24–30 repaired sites/10^{10} Da were detected in these experiments. Therefore, even assuming complete repair of dimers during this time period, the majority of lesions repaired were not of dimer origin. Hence, it is possible that the average patch size calculated reflects a combination of the long patches involved in dimer repair and the short patches that result from the removal of non-dimer damages.

Zamansky *et al* [11] have hypothesized that persistent sites of DNA damage in patients with SLE may result in enhanced levels of anti-DNA antibodies and pathogenic immune complexes containing DNA. In addition, investigators have recently begun to re-examine the possibility that the development of pathogenic autoantibodies against DNA may be an antigen-driven immune response [40]. Although native DNA has traditionally been considered to be a very poor antigen, evidence has been presented that bacterial DNA may indeed elicit an immune response [41]. Nucleic acid isolated from the sera of SLE patients is also immunogenic [42]. This antigenicity may be dependent upon unusual structures in the bacterial DNA because the secondary structure of DNA is known to be an important factor in defining the specificity of anti-DNA antibodies [43,44]. In addition, a role for a circulating DNA-protein complex in stimulating anti-DNA antibody production has been proposed [45]. It is therefore of interest to note that mice immunized with UV-irradiated DNA develop immune complex deposits at the dermal-epidermal junction upon subsequent exposure to UV radiation [46]. Pre-immunized animals also develop glomerular deposits of immune complexes upon administration of irradiated DNA [47,48]. Thus it appears possible that exposure to sunlight may result in the formation of structural alterations that enhance the antigenicity of DNA. An inability to properly repair these lesions may result in prolonged presence of antigenic DNA, higher levels of immune complexes, and greater tissue damage in SLE patients. Experiments are currently in progress to further define the abnormal responses of SLE cells to simulated sunlight with a goal toward developing an understanding of the relationship between these responses and the immunopathogenicity of SLE.

Drs. Susan Wallace and Nahum Duker are thanked for the gift of purified E. coli *endonuclease III.*

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